Enjoying the warming Mediterranean: Transcriptomic responses to temperature changes of a thermophilous keystone species in benthic communities.

Rocío Pérez-Portela [1, 2 *], Ana Riesgo [3], Owen S Wangensteen [4], Creu Palacín [1], Xavier Turon [2]

[1] Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona, and Research Institute of Biodiversity (IRBIO), Barcelona, Spain

[2] Center for Advanced Studies of Blanes (CEAB, CSIC), Blanes, Girona, Spain

[3] Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

[4] Norwegian College of Fishery Science, UiT The Arctic University of Norway, Tromsø, Norway.

* Corresponding author:

Rocío Pérez-Portela

Department of Evolutionary Biology, Ecology and Environmental Sciences, University of Barcelona,

643 Diagonal Avenue, 08028 Barcelona (Spain)

Phone: +34 934 021 439

Fax: +34 934 035 740

Email: rocio_perez@ub.edu

Running title: Transcriptomic response to thermal changes

ABSTRACT

Information about the genomic processes underlying responses to temperature changes is still limited in non-model marine invertebrates. In this sense, transcriptomic analyses can help to identify genes potentially related to thermal responses. We here investigated, via RNA-seq, whole-transcriptomic responses to increased and decreased temperatures in a thermophilous keystone sea urchin, *Arbacia lixula*, whose populations are increasing in the Mediterranean. This species is a key driver of benthic communities' structure due to its grazing activity. We found a strong response to experimentally induced cold temperature (7ºC), with 1,181 differentially expressed transcripts relative to the control condition (13ºC), compared to only 179 in the warm (22ºC) treatment. A total of 84 (cold treatment) and 3 (warm treatment) Gene Ontology terms were linked to the differentially expressed transcripts. At 7ºC the expression of genes encoding different heat shock proteins (HSPs) was up-regulated, together with apoptotic suppressor genes (e.g. *Bcl2)*, genes involved in the infection response and/or pathogen-recognition (e.g. echinoidin) and ATP-associated genes, while protein biosynthesis and DNA replication pathways were down-regulated. At 22 ºC neither HSPs induction nor activation of the previously mentioned pathways were detected, with the exception of some apoptotic-related activities that were up-regulated. Our results suggest a strong transcriptional response associated with low temperatures, and support the idea of low water temperature being a major limitation for *A. lixula* expansion across deep Mediterranean and northerner Atlantic waters.

Keywords: Transcriptomics, RNA-seq, warming, benthic species, Mediterranean, thermal responses

INTRODUCTION

Predicting organismal responses to environmental shifts is one of the main priorities of contemporary ecology (Calosi et al., 2017; King, McKeown, Smale, & Moore, 2018; Donelson et al., 2019). During the last decades, scientific studies have linked global warming, characterised by both an increase in mean temperatures and frequency of heat waves (Jordà, Marbà & Duarte 2012; Oliver et al., 2018), to detrimental impacts on marine systems at different biological levels. At the ecosystem level, impacts include the alteration of the whole ecosystem functioning as changes in food-wed dynamics and in ecosystem productivity occur, together with biodiversity loss (see Smale et al., 2019; Stillman, 2019). Mean temperature increases and heat waves also result in a number of lethal and sub-lethal effects on particular species and populations, including coral reef bleaching, alteration of animal migration routes and behaviour, and shifts of marine taxa distribution patterns, among many others (e.g. Hoegh-Guldberg & Bruno, 2010; Deutsch, Ferrel, Seibel, Pörtner & Huey, 2015; Hughes et al., 2017; King et al., 2018). At the individual level, when organisms are exposed to sub-lethal extreme temperatures stress is likely to occur (e.g. Buckley & Huey, 2016). To compensate the negative impacts related with stress, organisms develop different molecular and cellular mechanisms to maintain physiological performance and cell homeostasis (Pörtner, 2002; Buckley & Huey, 2016).

The cellular and molecular pathways involved in thermal stress response in marine organisms have been recently thoroughly studied. These studies showed changes in expression patterns of stress-responsive genes, including gene pathways that regulate metabolism, oxidation-reduction processes, cell cycle, and protein folding repair systems, among others (e.g. Gleason & Burton, 2015; Zhu et al., 2016; Gierz, Forêt & Leggat, 2017; Kim, Kim, Choi & Rhee, 2017; Xu, Zhou & Sun, 2018; Zheng et al., 2019). But, in marine invertebrates, most interest has focused on Heat Shock Proteins (HSPs) (Feder & Hofmann, 1999; Tomanek, 2010; Kim et al., 2017). HSPs are chaperones highly conserved during metazoan evolution that help proteins' folding and transport across cell membranes during non-stressful conditions. They also refold and stabilise denatured proteins under different conditions of stress (e.g. Matranga, Toia, Bonaventura & Müller, 2000; Di Natale et al., 2019). Nevertheless,

81 additional knowledge on the involvement of different genes and gene pathways, such as antioxidant
82 genes, apoptosis-associated and immune-associated genes, in ecologically relevant marine
83 invertebrates under thermal shifts is still desirable to unravel the whole mechanisms of thermal stress
84 response (Gleason & Burton, 2015; Zhu et al., 2016). One relatively recent approach to investigate
85 organismal rapid responses to environmental shifts, to identify potential physiological networks, and
86 to discover candidate genes involved in their responses, is to explore the whole transcriptional profiles
87 using RNA-seq techniques (e.g. Zhu, Zhang, Li, Que & Zhang, 2016; Evans, Pespeni, Hofmann,
88 Palumbi & Sanford, 2017; Xu et al., 2018). Although the relationship between mRNA transcript
89 abundance and protein abundance is still not clear (Feder & Walser, 2005), some studies have shown a
90 correlation between these two variables (Maier, Güell & Serrano, 2009). Changes in gene expression
91 are considered to be sensitive indicators of stress and potential predictors of organismal physiology
92 under experimental conditions (Feder & Walser, 2005; Buckley, Gracey & Somero 2006; Schoville,
93 Barreto, Moy, Wolff & Burton, 2012).

94 Among marine ecosystems, one the most impacted seas in the world is the Mediterranean
95 (Lejeusne, Chevaldonné, Pergent-Martini, Boudouresque & Pérez, 2010; Coll et al., 2010). This sea is
96 a hotspot of marine diversity subject to intense anthropogenic pressures (Claudet & Fraschetti, 2010;
97 Templado 2014), which interact with the ongoing global warming (Francour, Boudouresque,
98 Harmelin, Harmelin-Vivien & Quignard, 1994; Jordà et al. 2012). During the last three decades,
99 summer surface temperature (SST) has risen in the Mediterranean at a rate ranging from 0.25ºC per
100 decade in the western basin to 0.65ºC per decade in the eastern one (Marbà, Jordà, Agustí, Girard &
101 Duarte, 2015). High-resolution ocean models, considering a diversity of potential climate change
102 scenarios, have projected in all cases a significant increase in SST by the end of the century (see
103 Somot, Sevault & Déqué, 2006; 2008; Parry, Canziani, Palutikof, Van Der Linden, & Hanson, 2007;
104 Shaltout, & Omstedt, 2014). A Mediterranean seawater temperature rise represents a challenge for
105 most mediterranean taxa, reflected in sub-lethal effects linked to behavioural and physiological
106 responses (e.g. Anestis, Lazo, Pörtner & Michaelidis 2007; Prusina et al., 2014), lethal outcomes,
107 including mass mortality events associated to heat waves (e.g. Cerrano et al., 2000; Coma et al., 2009;

Garrabou et al., 2009), and the collapse of whole ecosystems along the warmest areas of the Mediterranean (Rilov, 2016). This warming also brings about other indirect effects, accelerating the entrance of warm-water alien species (Raitsos et al., 2010) and promoting the expansion of subtropical species that naturally colonised the Mediterranean during different geological periods (Briand, 2008; and examples of echinoderms in Wangensteen, Turon, Pérez-Portela & Palacín, 2012; Garcia-Cisneros et al. 2017; Pérez-Portela et al., 2019).

The black sea urchin *Arbacia lixula* (Linnaeus 1758) has tropical affinities (Tortonese, 1965) and an amphi-Atlantic distribution across shallow rocky ecosystems, being the Moroccan coast its northern-most distribution limit in the east Atlantic. This sea urchin entered the Mediterranean basin during the last Pleistocene interglacial period (Wangensteen et al., 2012; Pérez-Portela et al., 2019), and it is now a common species across the whole Mediterranean (Tortonese, 1965; Palacín, Turon, Ballesteros, Giribet & López, 1998). Densities of this species significantly increased in some Mediterranean areas during the last decades (Francour et al., 1994, Harmelin et al., 1995; Hereu et al., 2012), and it is among the key drivers structuring littoral communities due to its grazing activity (Bonaviri, Fernández, Fanelli, Badalamenti & Gianguzza, 2011). The species is capable of shifting littoral complex macroalgal beds into "barren grounds"- areas of high densities of sea urchins deprived of erect seaweeds and dominated by crustose coralline algae - (Gianguzza et al., 2011; Bonaviri et al., 2011). Several authors have predicted that the foreseen global warming might have a positive effect on its reproduction output and larval survival (Francour et al., 1994; Gianguzza et al., 2014; Wangensteen, Dupont, Casties, Turon & Palacín, 2013a; Wangensteen, Turon Caso & Palacín 2013b; Visconti et al., 2017). This potential effect, if real, will represent a worrisome increase of the impact of this sea urchin on littoral ecosystems in a near future (Gianguzza et al., 2011; Wangensteen et al., 2013a, 2013b). On the other hand, it seems that the distribution of *A. lixula* is constrained by low temperatures, like the low sea surface temperature provoked by the southward Portugal Current (Martins, Hamann & Fiùza, 2002), which might be the cause of its absence along the south Atlantic coast of Europe (Wangensteen et al., 2012). In this sense, experiments to investigate the potential of *A. lixula* to invade deep seawaters, analysing the combined effect of pressure (from 1 atm to 250 atm)

and temperature (from 5ºC to 15ºC) on the survival of embryos and larvae, showed that the combination of high temperatures and pressures, rather than temperature *per se*, might be the major factor limiting the distribution in depth (Young, Tyler & Fenaux, 1997). It was then concluded that *A. lixula* is more likely to invade deep habitats of the Mediterranean than open Atlantic ones, the latter being characterised by lower deep temperatures (Young, Tyler & Fenaux, 1997). In contrast, more recent studies have demonstrated higher mortality rates, larval growth abnormalities and significant delays in settlement at the lowest experimental temperatures tested in this species (experimental temperatures from 18ºC to 22ºC in Privitera, Noli, Falugi, & Chiantore, 2011; and from 16ºC to 19ºC in Wangensteen et al., 2013a). According to these studies, the abundance of *A. lixula* in the Mediterranean might be constrained by the low winter temperature of colder years, when mean temperatures can drop to 11°C, because gonad maturation is then considerably impaired (Lejeusne et al., 2010; Wangensteen et al., 2013a). But whereas the mentioned studies shed some light on the effects of thermal variation on the early development stages of *A. lixula*, almost nothing is known about its effects on the general performance of adult individuals, which can have different thermal sensitivity (Buckley & Huey, 2016). The capability of adult individuals to acclimatise and endure thermal changes is highly relevant from an evolutionary perspective. It not only affects their own survival and/or fertility, but can also result in negative transgenerational carry-on effects on hatchability and larval size of the next generation, which have been shown after prolonged periods of parental exposure to elevated temperatures in some sea urchins (Zhao et al., 2018). In sea urchins, transcriptomes from different tissue types and larval thermal stress responses have been characterised (e.g. Runcie et al., 2012; Gillard, Garama & Brown, 2014; Gaitán-Espitia, Sánchez, Bruning & Cárdenas, 2016; Pérez-Portela, Turon & Riesgo, 2016; Jia et al., 2017; Clark et al., 2019). But, to our knowledge, transcriptome-wide screenings have never been used for measuring responses to thermal variation in adult individuals of this animal group.

The aim of this study is to explore the short-term transcriptional response to thermal changes in the subtropical sea urchin *A. lixula*. We set three specific objectives for our study: a) To quantify and compare transcriptional responses to both high and low temperature treatments in *A. lixula* under

experimental conditions, b) To identify some of the most important candidate genes involved in rapid thermal responses in sea urchins, and c) To determine the conservation of the genetic machinery involved in thermal responses for both increase and decrease temperature challenges.

Many studies on global warming focus on the negative effect of rising temperatures, but in this study, we worked under the hypothesis that *A. lixula* will experience higher stress when subjected to low rather than to high temperatures. Based on previous transcriptional information from marine invertebrates under thermal stress (e.g. Gleason & Burton, 2015; Zhu et al., 2016), we also expect changes of expression patterns in different gene pathways during our temperature treatments, including genes encoding HSPs, apoptosis and anti-apoptosis mechanisms, ATP-associated genes due to an increase of energy demand to restore cell homeostasis, antioxidant genes since extreme temperatures can increase cells' oxidative stress, and immune-associated genes (Xu et al., 2018). The information obtained here will be relevant to understand the ecophysiological patterns of sea urchins exposed to thermal challenges. We also discuss the significance of our findings for the foreseeable ecological spread of this keystone species in the Mediterranean.

MATERIAL AND METHODS

Sea urchin collection

Adult specimens of *A. lixula* were collected by SCUBA diving in December 2012 from the shallow subtidal population (5-8 m depth) of Punta Santa Anna, Blanes (41°40′22.47″N, 2°48′10.81″E, North-western Mediterranean; Figure 1). Specimens were quickly transported to the laboratory (less than 2 Km away) in a cooler with seawater and oxygen tablets to keep stress induced by land transportation to a minimum. Experiments were performed in the LEOV (Laboratory of Experimentation with Living Organisms) facility of the Centre for Advanced Studies of Blanes (CEAB), equipped with an open system of running seawater coming directly from a sea intake. Once in the laboratory, sea urchins were measured with callipers and left to adjust for 48 hours in a common chamber with airflow and

flow-through running seawater at 13˚C, which was the sea temperature in Blanes at the collection time. During these 48 hours animals had rocky surfaces available for grazing.

Experimental design

To quantify rapid transcriptomic responses of *A. lixula* under thermal assays, we exposed adult sea urchins (test diameter 40 to 50 mm) to three different treatments under controlled conditions in laboratory for 20 hours: control (CT) with sea water at 13˚C± 1˚C, sea water temperature at 7˚C± 0.5˚C (T7), and sea water temperature at 22˚C ± 0.5˚C (T22). We set the temperature exposure time to 20 hours because previous experiments of thermal stress responses in other marine invertebrates demonstrated maximum peaks of expression between the first 6- 24 hours, depending on the genes (e.g. Zhu et al., 2016; Kim et al., 2017).

It is important to note that our goal was to submit the test organisms to an acute thermal change to measure their responses, not to mimic highest or lowest seasonal temperatures in the area. The treatment temperatures were chosen to represent an important shift with respect to the controls (13ºC, the surface water temperature at this location when sea urchins were collected in wintertime) while remaining within realistic values for our area of study, the NW Mediterranean. Thermal sensitivity and resistance of organisms are not constant over time and often shift in response to seasonal conditions (Buckley & Huey, 2016). The temperatures chosen, therefore, would have been different had we performed the trials at other seasons as they were contingent on current conditions at the time of the experiment. The average summer surface temperatures in the Mediterranean range from 22ºC to 28ºC, with the lowest values at the north Aegean, Alboran Sea, and NW Mediterranean (Pastor 2012; Marbà et al., 2015). The global average for the coldest month of the year (February) in the Mediterranean is 14.5ºC, with a lower average value (12º-13ºC) found at the NW Mediterranean (Pastor 2012) (see Supplementary Information S1). Since the species' thermal history can determine the thresholds of stress response (Osovitz & Hofmann 2005) and thermal sensitivity can change over the seasons, we made a preliminary assessment of the tolerance limits of our NW Mediterranean

population at that time of the year (so-called here "trials"), with several temperatures assayed over a 20 hours period and visual inspection of the state and activity level of 10 sea urchins per temperature treatment. Specimens used for the trials were not used for further experiments and were returned to the sea after experimentation, nor were samples collected for transcriptomic analysis during the trials. For the trials, we used 22ºC, 24ºC, and 26ºC as upper thermal limits, and 12ºC, 9ºC and 7ºC as lower limits. 7˚C and 22˚C marked the lower and upper thresholds, respectively, at which all individuals used for the trials remained alive, visually healthy (intact skin, no algae or microorganism colonies growing up over the animal surface and no massive spine lost) and active (feet and spines movement). For the cold treatment, 7ºC (a decrease of 6ºC relative to the control) was the limit temperature achievable in winter in shallow embayments in the NW Mediterranean (e.g. Ordoñez et al. 2015), while for the warm treatment we increased temperature by 9ºC (relative to the control), being 22º-23ºC the conditions encountered in mid-summer in the study area (e.g. Pastor 2012; Marbà et al., 2015; De Caralt, González, Turon & Uriz, 2018). Over 22ºC, experimental animals either died or presented clear signs of infection with microorganism colonies over the skin and/or massive loss of spines. We emphasize that, while sea urchins thrive at this temperature and higher in summer, we were performing an acute exposure treatment during wintertime, so we had to adjust our treatments accordingly.

Our experimental design for transcriptomic analysis consisted of two different experiments: A "Low temperature" experiment comparing the control condition at 13˚C ± 1˚C and experimental condition at 7˚C ± 0.5˚C, hereafter named as "Control vs T7", and a "High temperature" experiment comparing the control condition at 13˚C ± 1˚C and experimental condition at 22˚C ± 0.5˚C, hereafter named as "Control vs T22" (see Figure 1). Samples used as control condition were the same for both experiments, since all treatments were run at the same time and laboratory. After the acclimation period of 48 hours, each sea urchin was placed in an independent aquarium to avoid interactions among specimens. Each aquarium had constant airflow and the seawater temperature was set at the required temperature (13ºC, 7ºC or 22ºC) prior to adding the sea urchins. Temperature of the aquaria was controlled with HOBO loggers (one per aquarium). Aquaria with different treatments were

randomly allocated across the wet-lab space to avoid any bias related to their spatial distribution. Animals were not fed during the 20 h of the experimental time, and seawater pH (8.1) was monitored during the experiments. Eight different replicates (specimens) per treatment were included, although for gene expression analyses only six of them were processed. The sample size of 8 was used to ensure an even proportion of sexes in the specimens analysed (since sex determination can be only performed *a posteriori* after dissection), and indeed we processed for transcriptomic analyses 3 males and 3 females per treatment. After the 20 hours of treatment, sea urchins were removed from the aquaria, quickly dissected under RNAase free conditions, and coelomocyte fluid collected and processed as explained in the next section.

For sex determination we used histological techniques. One gonad per individual was obtained and preserved in 4% formaldehyde. Gonad samples were washed in distilled water, dehydrated, embedded in paraffin, cut in 5 μm sections using a Microm HM325 Microtome, and stained in haematoxylin–eosin as described in Wangensteen et al. (2013b) and Garcia-Cisneros et al. (2017). Sex was then determined under the optical microscope.

Coelomocytes collection and RNA sequencing

Coelomocytes consist of several cell types contained in the coelomic fluid and are immune effectors in echinoderms (Matranga et al., 2000; Smith et al., 2018). They have been used as biomarkers of stress due to their prompt response to changing environmental conditions (Matranga et al., 2000, Matranga, Bonaventura & Di Bella, 2002; Matranga et al., 2005; Pinsino et al., 2008) that can reduce the protective capacity of these cells and rapidly induce activation of the heat shock proteins expression (Matranga et al., 2000; Pinsino et al., 2008). Additionally, these cells showed higher thermal response capacity than other tissues in sea urchins (e.g., digestive tissues, Gonzalez et al., 2016), and protocols for extraction of high quality RNA and high throughput sequencing have been developed for this tissue type in *A. lixula* (Pérez-Portela & Riesgo 2013; Pérez-Portela et al., 2016).

Five millilitres of the coelomic fluid of each specimen (a total of 18 specimens; six per

treatment) was collected using a sterile syringe inserted through the peristomic membrane, taking care not to puncture the gut. The fluid was then centrifuged, and all fresh cellular components (coelomocytes) gathered and quickly embedded in TRizol reagent (Invitrogen, www.invitrogen.com). Total RNA was directly extracted from coelomocytes following a protocol previously optimized for this species (Pérez-Portela & Riesgo 2013 and Pérez-Portela et al., 2016). Integrity of total RNA and potential DNA contaminations were initially evaluated by visualizing the 28S rRNA and 18S rRNA bands into a 1% agarose gel in 1x TAE Buffer. Concentration of the RNA extracts was assessed in a Hellma spectrophotometer (Hellma Analytics), and total RNA extracts were also run in an Agilent 2100 Bioanalyzer (Agilent Technologies) at the Scientific and Technical Services of the University of Barcelona for quality measurements. High quality RNA (RINs over 8.5) samples were sent to the National Centre of Genomic Analyses of Barcelona (CNAG) for mRNA isolation, cDNA library construction, normalization and sequencing.

Isolation of mRNA and cDNA library preparation for each of the 18 specimens were performed using the Illumina TruSeq RNA Sample Prep Kit (Illumina, Inc.) following the manufacturer's recommendations, with an input of 800-900 ng of mRNA, and average insert size of the libraries of 300 bp. Quality and concentration of the 18 cDNA libraries was controlled with Ribogreen Assays in a NanoDrop 3300™ Fluorospectrometer (Thermo Fisher Scientific, www.thermofisher.com). The 18 libraries (6 per treatment) were multiplexed with Illumina barcodes, 5 libraries per lane were sequenced on an Illumina HiSeq2000 Sequencer, and 101 base paired-end reads were generated. The 18 libraries from different treatments were randomised across Illumina lanes.

Sequence processing and de novo *assembly*

The software FASTQC v. 0.10.0 (www.bioinformatics.babraham.ac.uk) was used to visualise and measure the quality of the raw reads generated in the HiSeq2000. Adapters and bases with low quality (phred scores <33) were trimmed off, and a length filter was applied to keep only sequences of >25

bases using TrimGalore v. 0.2.6 (www.bioinformatics.babraham.ac.uk). High-quality reads were re-screened in FASTQC to ensure a good quality of the samples after trimming. A basic scheme of the most important steps of our pipeline is presented in Figure 2.

Two *de novo* assemblies, hereafter named as "CT+T7" and "CT+T22", one per experiment ("Control *vs* T7" and "Control *vs* T22", respectively), were separately built up as reference for gene expression analyses. Due to technical difficulties and the low quality of two libraries, for gene expression only 5 samples could be used for each of the T7 and T22 treatments (see details in Results section and Figure 1). Nevertheless, these two libraries discarded for gene expression could be used for the assembly of the respective references. The assemblies were performed separately for each experiment to ensure that the corresponding reference is the most comprehensive for each particular experiment. The *de novo* assemblies were performed with the software Trinity (Grabherr et al., 2011), which allows detecting differentially spliced isoforms, with default parameters for this software. Only contigs with a minimum length for reported transcripts of 200 bp and at least 10x coverage were retained for the assemblies.

The two *de novo* assemblies were separately blasted against a selection of the *nr* database of NCBI containing only proteins from Metazoa (blastx) using BLAST (Altschul et al., 1997) with a cut-off E value of $1e^{-5}$. The highest scoring blast hit was used to assign a gene name to each contig. *De novo* assemblies were also blasted against both a database containing proteins of bacteria (blastx), and a database of ribosomal DNA of bacteria (blastn) obtained from NCBI to remove bacterial contaminations. Sequences with blast hit exclusively against proteins and nucleotides of bacteria were eliminated from the datasets.

Blast results against Metazoa served as a database for annotation of transcripts differentially expressed between treatments (see below). Moreover, Blast results of the assemblies were used to retrieve Gene Ontology (GO) terms with BLAST2GO (Conesa et al., 2005) under different categories: biological processes, molecular function and cellular component, which are hierarchically organized into different levels (see Figure 2). The completeness of the reference transcriptomes was assessed

with BUSCO (Benchmarking Universal Single-Copy Ortholog) against the eukaryotic and metazoan databases (Simão, Waterhouse, Ioannidis, Kriventseva & Zdobnov, 2015).

Differential expression analyses and annotation

Reads from all replicates in each experiment were aligned against the corresponding "reference" transcriptome as per experiment (see Figure 2). Paired reads after trimming were mapped using Bowtie2 v. 2.2.1 (Langmead & Salzberg, 2012) as implemented in Trinity (Grabherr et al., 2011). RSEM v. 1.2.11 (Li & Dewey, 2011) was then run to generate a table with read counts, and unmapped reads were discarded. In the "reference" transcriptomes, transcripts of the same trinity component were treated as different isoforms. We retained information of differential expression of all isoforms detected for a given gene (or component) because they may have different functions.

Differential expression (DE) analyses of the two experiments were performed with the package DESeq2 (Love, Huber & Anders, 2014) in R v 3.2.1 (R Development Core Team 2008). Before performing the analyses, preliminary tests to investigate differences in gene expression between sexes and treatments were performed. No significant differences in response to treatments were observed between males and females, and "sex" was not considered as a variable in further analyses.

Before analysing differential gene expression, read counts were normalized, and then a negative binomial model was fit to accurately estimate differential expression. The significance value for multiple comparisons was adjusted to 0.01 with the function "padj" (Benjamini-Hochberg adjustment) as implemented in DESeq2. Transcripts with significantly different expression values relative to the controls will be hereafter called "DE" transcripts. Component Analyses (PCAs) were performed and plotted with the same package to visualize variation of expression levels among samples and treatments. Visualization of the significant outcomes of isoforms differentially expressed (up- and down-regulated) between treatments of each experiment was obtained with a heatmap performed with the "gplots" package of R (Warnes, Bolker, Bonebakker & Gentleman, 2016).

Using the GO annotation results from the *de novo* assemblies of the two experiments, we obtained the GO terms associated to the differentially expressed isoforms, which were then input (together with their associated log2foldchange) to the REVIGO web server (Supek, Bošnjak, Škunca, & Šmuc, 2011) to obtain summaries of GO terms. Results were graphically represented with the "treemap" R package. Size of the rectangles was adjusted to reflect the log2foldchange in REVIGO. Differentially expressed isoforms without blast hit, unknown function and/or without annotation for each experiment were assessed with the InterProScan 5 software (Jones et al., 2014), which predicts protein family membership and the presence of functional domains and sites, at the Superfamily level (De Lima Morais et al., 2011). The InterproScan was run as implemented in the Blast2GO software with default parameters. We finally merged the results of the associated GO terms and those from InterProScan with the purpose of increasing our knowledge of coelomocyte gene functions and GO annotations.

In order to identify common genes and/or isoforms differentially expressed under temperature increase and decrease, the *de novo* assemblies of both experiments, that assigned different transcript names to all isoforms, were blasted against each other using BLASTn.

RESULTS

Data filtering and de novo *assembly*

A total of 18 RNA-seq datasets were used for *de novo* assembling (see Figure 2), and 16 datasets for quantifying transcriptomic responses in *A. lixula* (see Figure 1) since one sample from experiment T7 and another from T22 were discarded for gene expression analyses because of their low quality. Datasets have been deposited in Mendeley Data (doi.org/10.17632/5673n552yj.1). The number of trimmed reads used for *de novo* assembly, as per sample replicate and treatment, are detailed in Tables 1 and 2. All replicates had over 26 million reads.

The *de novo* assembly "CT+T7", used as a reference for the "Control *vs* T7" experiment,

included 141.5 Megabases that rendered 211,650 transcripts (including both genes and their different isoforms), and 19.6% of them had blast hit with known proteins of metazoans (see species blast hit distribution in Supplementary Information S2). The reference assembly "CT+T22" for the "Control *vs* T22" experiment included 147.4 Megabases, and rendered 219,655 different transcripts, from which 17.9% had blast hit (see species blast hit distribution in Supplementary Information S2). Both *de novo* assemblies were very comparable (and had 99,5% transcripts in common), presenting relatively high N50 values, between 1,102 and 1,114, meaning that over 50% of the transcripts were longer than 1,100 bases. Details of the *de novo* assemblies for the two different experiments are presented in Table 1. Both, "CT+T7" and "CT+T22", showed high completeness when compared with BUSCO conserved ortholog databases of eukaryotes and metazoans (see Table 3). For the reference assemblies, "CT+T7" and "CT+T22", 194 and 4,293 transcripts, respectively, had blast hits against proteins and/ or nucleotides of bacteria and were removed from subsequent analyses. In fact, most differences between the reference assemblies "CT+T7" and "CT+T22" were due to the amount of bacterial transcripts.

General results of differential expression analyses

The differential expression analyses revealed changes in gene expression between controls and temperature treatments in both experiments, "Control *vs* T7" and "Control *vs* T22", but with a remarkable difference in the magnitude of the transcriptomic responses, which was over 6 times higher in number of differentially expressed (DE) transcripts in the former experiment, as explained below. We also detected differences in gene expression among different isoforms of the same genes.

In the "Control *vs* T7" experiment, we detected 1,181 DE transcripts between CT and T7, being 720 transcripts up-regulated at T7 (61% of the total DE transcripts) and 461 transcripts down-regulated at T7 (49% of the total DE transcripts) (see Figure 3). 445 transcripts (37.7 % of the total DE transcripts) had blast hit and known function (see Table 4), including 28 transcripts within the top 50

most significant DE (see Supplementary Information S3). Regarding different isoforms, over all DE transcripts (potential genes), 176 presented different isoforms (see Supplementary Information S3). Ten genes presented all isoforms DE between CT and T7, whereas the other 166 genes only showed some of their isoforms differentially expressed between treatments.

The number of DE transcripts in the "Control *vs* T22" experiment was much lower than in the "Control *vs* T7" experiment, with only 179 DE transcripts, being 57 transcripts up-regulated (32% of the total DE transcripts) and 122 transcripts down-regulated (68% of the total DE transcripts) in the T22 treatment (see Figure 2). Only 35 transcripts (19.7 % of the total DE transcripts) had annotation and known function (Table 4), 10 of them within the top 50 most significant DE. Of these 35 transcripts, 27 had different isoforms, and in all cases only one of their isoforms was DE between CT and T22 (see Supplementary Information S3). A complete list of differentially expressed, annotated transcripts for both experiments is presented as Supplementary Information (S3), including transcript identification code (id), logarithm of the fold change, adjusted *p*-value with FDR correction obtained from the expression analyses, gene description, number of isoforms found and transcripts with known function within the top 50 most significant DE (* Top 50 DE). Fourteen DE transcripts were common between experiments (see Figure 3) and most of them featured opposite responses between treatments. Only four of these transcripts had annotations (*fam-55cc*, *tripartite motif-containing protein* 3, and *wsc domain- containing protein 1* with opposite responses in T7 and T22, whereas the *histone-lysine n-methyltransferase prdm 9* was down-regulated in both temperature treatments of the two experiments, T7 and T22).

Figure 4 represents the hierarchical clustering of all transcripts related to their expression differences between treatments for each experiment (heatmaps), and Figure 5 the corresponding PCAs. The heatmaps and PCAs showed, in general, little differentiation between replicates of the same treatment, and large differences in transcript expression between treatments. Only one of the control replicates had a mixed pattern of expression between that of the other control samples (Control replicate 1, see Figure 4) and those from treatment 22ºC, and clustered together to the T22 samples on the PCA (see Figure 5). However, this control sample did not follow the same trend in the other

experiment, “Control *vs* T7”.

A total of 84 and three GO terms were found associated to the differentially expressed transcripts in the “Control *vs* T7” and “Control *vs* T22” experiments, respectively (Table 4). The InterProScan could only predict information of protein domains in six uncharacterised transcripts of the “Control *vs* T22” experiment. In Figures 6 and 7 the up- and down-regulated GO categories associated to DE transcripts from the two experiments are depicted. These GO terms were not equally represented among categories between up- and down-regulated DE transcripts, or between experiments. For the experiment “Control *vs* T7” the most important up-regulated GO term categories for Biological Process (BP) were “tyrosine metabolism” (including “positive regulation of apoptotic process”), “peptidyl-tyrosine dephosphorylation”, “protein folding” and “ATP hydrolysis coupled proton transport”; “proton-transporting V-type ATPase-V0 domain” and “sarcoplasmic reticulum” for Cellular Component (CC), and “GTP binding”, “protein tyrosine phosphatase activity”, “Protein tyrosine phosphatase activity”, “sulfo-transferase activity”, “hydrogen ion transmembrane transporter activity” and “lipid binding” (among others) for Molecular Function (MF) (Figure 6). The most important down-regulated GO categories for BP were “neurotransmitter transport”, “Intracellular signal transduction” and “protein O-linked glycosylation”; “nuclear origin of replication recognition complex”, “cell”, “intracellular” and “integral component of membrane” for CC, and “protein-N-acetylglucosaminyltransferase activity”, “sequence- specific DNA binding”, NAD-dependent histone deacetylase activity” and “zinc ion binding” (among others) for MF (Figure 6). For the experiment “Control *vs* T22” only GO information for down regulated transcripts could be obtained and, among them, the most important DE categories were “notch signalling pathway”, “multicellular organismal development” for BP, “integral component of membrane”, “membrane”, and “SAGA-type complex” for CC, and “calcium ion” and “protein binding” for MF.

Differentially expressed genes involved in thermal stress, apoptotic processes and immune responses in Arbacia lixula

458 At 7˚C, the production of different heat shock proteins was up-regulated, including different
459 transcripts for the Heat Shock family proteins: an inducible *Hsp70*, and *Hsp71*, *Hsp90* and the *Dnaj*
460 *homolog subfamily c member 21(DNAJC21)*, which encodes a member of the DNAJ heat shock
461 protein 40 family (*Hsp40*) (see Supplementary Information S3 for *Hsp40* transcripts and foldchanges:
462 c256938_g1_i3, log2foldchange= 2.98; c260821_g2_i1, log2foldchange= 1.35; c260821_g1_i2,
463 log2foldchange= 3.05; c264479_g1_i1, log2foldchange= 7.35; c249691_g1_i1, log2foldchange= 1.91;
464 c271252_g1_i1, log2foldchange= 3.69) acting as a co-chaperone of *Hsp70* (Supplementary
465 Information S3). In addition, the receptor of stress *Wsc domain-containing protein 1* was found down-
466 regulated at 7˚C and up-regulated at 22˚C (Supplementary Information S3: c266025_g2_i1,
467 log2foldchange= -1.19; and c265343_g1_i1, log2foldchange= 1.84, respectively).
468 Several genes from the apoptotic gene complements were differentially expressed between
469 controls and T7. They included the *Bcl2* (up-regulated in T7, Supplementary Information S3:
470 c263429_g1_i1, log2foldchange= 2.17; and c271119_g2_i1, log2foldchange= 1.73), *sequestosome 1*
471 (up-regulated in T7, Supplementary Information S3: c257995_g1_i1, log2foldchange= 3.72) and *fas-*
472 *associating death domain-containing protein* and *death ligand signal enhancer* (down- and up-
473 regulated in T7, respectively; Supplementary Information S3: c268119_g1_i3, log2foldchange= -1.48
474 and c270362_g1_i1, log2foldchange= 1.99). In T22, we found upregulation of *immediate early*
475 *response 3-interacting protein 1-like* (Supplementary Information S3: c276658_g1_i2,
476 log2foldchange= 1.55).
477 At 7˚C, there was an up-regulation of genes involved in the innate immune response identified
478 as *echinoidin, senescence associated-gene* and *Tripartite motif-containing protein 3* (TRIM)
479 (Supplementary Information S3: c258741_g1_i1, log2foldchange= 5.35; c150071_g1_i1,
480 log2foldchange= 6.02; c273778_g2_i1, log2foldchange= 2.90). In addition, the genes *interleukin-17*
481 and cytohesin-like were also upregulated in T7 (Supplementary Information S3: c239836_g1_i1,
482 log2foldchange=6.22; and c263807_g1_i1, log2foldchange=1.77, respectively).
483
484 DISCUSSION

The response of marine organisms to thermal shifts is likely different across the species' range of distribution (Donelson et al. 2019). In our study, we investigated transcriptional responses of a keystone species, the black sea urchin, in the northern part of its range of distribution (NW Mediterranean). We found contrasting responses to low (7˚C) and high (22˚C) temperatures, with the former eliciting a much stronger reaction. Such differences were related to both the magnitude of the transcriptional response (e.g. number of up- and down- regulated transcripts and gene expression fold-change) and the diversity of genes and pathways involved in these responses.

The capacity of ectotherm species to thrive across wide temperature ranges is, in part, based on their ability to modulate the expression of genes encoding proteins involved in the physiological, metabolic and cellular stress responses (Stillman, 2003; Runcie et al., 2012; Tomanek, 2010; Kim et al., 2017). Resistance to acute sublethal temperatures is an adaptive trait that varies among species of the same genus from different latitudes and habitats (Stillman, 2003; Yao & Somero, 2012). In general, marine tropical species are more heat tolerant than their temperate and cold counterparts (Somero, 2010). Paradoxically, analyses of both marine and terrestrial ectotherms suggest that tropical, or the warmest-adapted species, may be more threatened by global warming because they live closer to their upper physiological thermal limit, and have higher metabolic rates that accelerate quicker than in colder species under rising thermal conditions (e.g. Stillman, 2003; Somero, 2010). According to this expectation, *A. lixula*, a heat tolerant species with sub-tropical affinities (Tortonese, 1965; Wangensteen et al., 2012), could be threatened by global warming across the warmest areas of its geographical distribution (Elmasry et al., 2015; Rilov 2015), where it might be closer to its thermal physiological limits. However, in the Northwestern Mediterranean this species is in the coldest part of their range of distribution, which encompasses both sides of the tropical and subtropical Atlantic (Wangensteen et al. 2012), and thus it could be more limited by cold temperatures. Current Mediterranean sea warming may be removing thermal limitations for this species (Francour et al., 1994; Gianguzza et al., 2014; Wangensteen et al., 2013a, 2013b; Visconti et al., 2017) allowing an increase in its abundance in the Mediterranean.

In general, it is difficult to determine whether changes of expression in particular genes have important functional consequences, because for each gene the threshold for metabolic and physiological downstream effects can be different, and relatively small changes in gene expression of only a few genes can be as functionally important as larger changes in other genes (Oleksiak, Roach & Crawford, 2005). However, the overall changes of gene expression patterns found in *A. lixula*, the number of genes differentially expressed, and the clustering of one control individual with the 22˚C experimental individuals at the PCA and heatmap, indicates a lower transcriptional response to rapid temperature increases in this subtropical species.

Decreasing temperatures elicited the activation of genes related to metabolism changes, pro- and anti-apoptotic mechanisms, and immune responses in coelomocytes of *A. lixula*. Among the upregulated genes related to the stress response at 7˚C, we detected the *Hsp71*, *Hsp90*, an inducible *Hsp70*, and *Hsp40*; being the last one a co-chaperone of the *Hsp70*. The protein *Hsp40s* stimulate the ATPase activity of *Hsp70s* and targets unfolded proteins to *Hsp70s* (Ngosuwan, Wang, Fung & Chirico, 2003). In general, these HSP chaperones are involved in the strong and mild thermal stress response and protein folding reaction to avoid protein denaturation in sea urchins, either in adult or early development stages and eggs (e.g. Matranga et al., 2000, 2002; Runcie et al., 2012; González, Gaitán-Espitia, Font, Cárdenas & González-Aravena, 2016). Their presence might be involved in the wide thermal distribution of some particular marine species (see Zhu et al., 2016, and references herein), and the HSP family seems to be a mechanism to cope with the stress associated with cold, and with temperatures existing along the lower-end of thermal tolerances for *A. lixula* (e.g. NW Mediterranean). On the other hand, no overexpression of genes encoding HSPs was detected at 22˚C in *A. lixula*.

Under conditions of thermal stress, protein refolding by HSPs may not be efficient enough, and misfolded protein degradation can be necessary to restore cell homeostasis (Mosser et al., 2000). Therefore, other mechanisms such as proteolysis to eliminate disfunctional proteins via the *Ubiquitin* proteosome pathway, and finally apoptosis to eliminate damaged cells, can be activated (Somero, 2010; Logan & Somero, 2011; Zhu et al., 2016). We only detected signs of *Ubiquitin* proteosome

539 pathway activation in the 7˚C treatment, with the up-regulation of the gene *sequestosome 1*
540 (Supplementary Information S3), which is an autophagosome cargo that detects proteins for autophagy
541 previously identified in echinoderms (Bitto et al. 2014), and the *e3 ubiquitin-protein ligase*, which
542 targets damaged proteins for transport and degradation by the proteasome (Ardley & Robinson, 2005).
543 In addition, we observed differential expression of several apoptosis-associated genes in both
544 treatments, 7˚C and 22˚C. Several studies demonstrated that sea urchins hold a complex apoptotic
545 system (Agnello & Roccheri 2010; Lesser, 2012). We found transcriptional changes at 7˚C in
546 apoptosis suppressor genes such as the *Bcl2* (up-regulated, Supplementary Information S3), widely
547 distributed in different marine invertebrates (see Lesser, 2012), and in genes containing death domains
548 (down-regulated: *fas-associating death domain-containing protein* and *death ligand signal enhancer*,
549 Supplementary Information S3) that induce cell apoptosis through the regulation of caspase activation
550 (Agnello & Roccheri 2010; Zhu et al., 2016). These findings suggest the activation of some particular
551 pathways to control the programmed cell death at low temperatures. The up-regulation at 22˚C of the
552 gene *immediate early response 3-interacting protein 1-like* (Supplementary Information S3), which is
553 a molecule involved in protein transport between the Sarcoplasmic reticulum and Golgi apparatus and
554 that mediates apoptosis in human cells (https://www.uniprot.org), suggests that apoptosis is also
555 occurring as a response of increased experimental temperatures.
556 Additionally, a *Serine threonine- protein kinase pim3*, an enzyme involved in the regulation of
557 cell transport and survival, which prevents apoptosis by inducing the release of the anti-apoptotic *Bcl2*
558 mentioned before (Cross et al., 2000) was also overexpressed at 7˚C, whereas a *Serine threonine-*
559 *protein phosphatase 6*, with opposite function to the kinase enzyme (Cross et al., 2000), was down-
560 regulated at 22˚C. Another interesting finding is the opposite pattern of gene expression found
561 between experiments for the *Wsc domain-containing protein 1* (down-regulated at 7˚C and up-
562 regulated at 22˚C) (Supplementary Information S3). Different members of the *Wsc* family are
563 identified as putative receptors of stress and required for the heat shock response and the maintenance
564 of cell wall integrity in yeasts (Lodder, Lee & Ballester, 1999). The *Wsc* members are upstream
565 regulators of other serine-threonine kinases, the protein kinase C1 (PKC1) and mitogen-activated

protein kinase (MAPK), which can promote apoptosis (Lodder et al., 1999; Cross et al., 2000). The differential expression of these molecules between control conditions, 7˚C, and 22˚C, evidences the different regulation systems of apoptosis and control of cell damage at different temperatures in *A. lixula*.

Previous experiments on echinoderms demonstrated the effect of thermal stress on the immune capacity of coelomocytes, being this effect greater at higher than lower temperatures in the analysed species (the sea cucumber *Apostichopus japonicus*, Wang, Yang, Gao & Liu, 2008). However, in *A. lixula*, it was the lowest temperature the one that triggered a higher immune response in terms of gene expression. The *echinoidin*, *senescence associated-gene*, *cytohesin-like* and *tripartite motif-containing protein 3* (TRIM) (Supplementary Information S3) involved in the infection response and/or pathogen-recognition process against bacteria, fungi and viruses (Smith et al., 2006; Ozato, Shing & Chang, 2008) were up-regulated at 7ºC. In addition, the gene *interleukin-17* (Supplementary Information S3) which is a cytokine inducing and mediates proinflammatory responses in metazoans and stimulates phagocytosis in echinoderms (Beck et al. 1993), was also up-regulated at 7˚C. None of these immune genes were, however, activated (or, when detected, were down-regulated) at the highest experimental temperature (e.g. TRIM), suggesting no immune response at 22˚C.

The differentially expressed genes for the low and high temperature experiment were associated to different GO categories that provide additional information. These GO categories summarise the most significant biological processes, cellular components, and molecular functions that were up- and down- regulated during the experimental response in *A. lixula*. For the high thermal stress experiment, we could only recover GO terms of three transcripts, and therefore, there is limited information to reach conclusions on the GO categories for this experiment. However, we detected the down-regulation of two interesting GO terms, the “Notch signaling pathway” with the associated gene *neurogenic locus notch* (*Notch1*), and the “integral component of membrane” with the associated gene encoding a Notch ligand, the *delta protein.* Notch is a cell signaling system calcium-dependent involved in different functions including cell differentiation, proliferation and apoptosis. In general,

Notch inhibits apoptosis and induces cell proliferation but, *in vitro* studies, using different cell lineages, showed that hyperthermia reduced *Notch1* expression and apoptosis in some cell lineages, whereas a opposite pattern was obtained in other cell lineages (Basile et al., 2007). Therefore, the effect of the Notch down-regulation at high temperatures in coelomocytes is not completely clear, but it suggest the existence of an alternative pathway of apoptosis under thermal stress.

Among the GO terms up-regulated during cold exposure that add further information we found the "Tyrosine metabolism" term, which is related to cell protection against stress, including the up-regulation of HSPs, cytoskeletal stabilization and apoptosis decrease (Baird, Niederlechner, Beck, Kallweit & Wischmeyer, 2013). This major GO term also includes the subordinate "Positive regulation of apoptotic process", which can induce apoptosis when protein refolding by HSPs is not efficient enough. The induction of HSPs during thermal stress can considerably increase the energy demand in cells (Tomanek 2010; Dong, Yu, Wang & Dong, 2011). This increased energy demand is reflected in the over-representation of the GO category "ATP hydrolysis", a catabolic process that releases energy previously stored in the form of ATP, and the up-regulation of the *V-type proton ATPase* gene (see Supplementary Information S3), a proton pump found within the "proton−transporting V−type ATPase, V0 domain" term. Likewise, the terms "Protein folding" and "Protein transport", the last one subordinate to the "ATP hydrolysis" category, are linked to protein transport to the Sarcoplasmic reticulum for folding reaction to avoid protein denaturalization by HSPs. Hence, the "Sarcoplasmic Reticulum" category, a key organelle involved in the thermal stress response that ensures that misfolded proteins are directed towards a degradative pathway to the central cytoplasmic proteolytic machinery (Malhotra & Kaufman, 2007), was also over-represented at 7ºC. Actually, the induction of expression of *Hsp70s* has been directly associated to the accumulation of unfolded proteins in the sarcoplasmic reticulum (Rachel, Tyson & Stirling, 1997; Rao et al., 2002), which are later eliminated if refolding fails by retrograde transport across the reticulum membrane (Kostova & Wolf, 2003). Other minor up-regulated GO terms, at the biological process and molecular function, were "oxidation-reduction processes" (1 Go term) and "oxidoreductase activitiy" (2 Go terms). These terms suggest that low temperature affects the intracellular redox state in coelomocytes.

Among the down- regulated GO terms at the 7˚C treatment we found "Neurotransmitter transport" with the associated differentially expressed genes *Creatine transporter* and *Trafficking protein particle complex subunit 2 protein*. The *Creatine transporter* is essential for normal brain function in humans and tissues with high energy demands because, together with other molecules, maintains ATP levels (Christie, 2007). The down-regulation of these genes and pathways could be a potential response to energy competition with the induction of HSPs during thermal stress. The 7˚C treatment also seemed to inhibit nuclear replication, as represented by the down-regulation of the "nuclear origin of replication recognition complex" and "DNA replication" terms, among others. The origin recognition complex is a ATP-dependent system that, among other factors, enables the initiation of DNA replication in eukaryotic cells (Li & Stillman, 2013). Cells under stressful conditions must prevent cell division in favour of protective funtions (Jonas, Liu, Chien & Laub, 2013), as well as to avoid entering in a new DNA replication cycle if there is DNA damage (Lee et al., 2009). We also found down-regulation of the "Intracellular signal transduction" term, with the subordinate "cell redox homeostasis" and "smoothened signalling pathway" terms, and the "protein O-linked glycosylation" term. Smoothened is a key transmembrane protein involved in a critical cell-to-cell communication system for tissue homeostasis. Glycosylation, on the other hand, is one of the most common post-transcriptional modifications during protein biosynthesis, which contributes to increase protein solubility and stability against proteolysis, and can also be involved in their correct folding (Shental-Bechor & Levy, 2008). Hence, the down-regulation of these last two terms reflects the potential negative effect of low temperatures on protein biosynthesis and stabilization, and homeostasis control in coelomocyte cells.

In summary, our results based on RNA-seq analyses of the whole transcriptome of coelomocytes in *A. lixula* show that this sea urchin, or at least this NW Mediterranean population (Wangensteen et al. 2012; Pérez-Portela et al. 2019), displays strong gene expression changes in response to the cold treatment, with activation of many genes whose functions could be related to stress responses in the form of chaperone production, apoptosis regulation, ATP-associated genes, enhancement of the immune system and redox processes, and down-regulation of gene pathways

related to protein biosynthesis and DNA replication. Nevertheless, contrary of that found in other studies (e.g. Gleason & Burton, 2015; Zhu et al., 2016) no activation of genes encoding antioxidant enzymes was detected in our experiments. As we initially expected, a markedly lower response is found in the warm treatment, with no activation o deactivation of the previously mentioned pathways, with the exception of the apoptosis regulation. Although some caution is needed, as we have characterized transcriptional changes and not protein levels, the differential patterns found in these genes strongly indicated that sea urchins are more stressed under lowered experimental temperatures.

We acknowledge that we have tested only acute thermal conditions, without any progressive acclimation. This is an unrealistic scenario but was chosen to elicit a short-term measurable response. This response was much more marked against lower than higher temperatures, which indicates potential to compensate for cold stress. Future research should investigate a wider panoply of temperature regimes combined with acclimation periods. However, our results indicate that *A. lixula* might require energy expenditure to withstand the stress associated with low temperatures, while it does not undergo relevant transcriptional changes when exposed to warm temperatures. This is coherent with the notion of a thermophilous species living near the colder limit of its physiological tolerance, as found also when analysing reproductive and larval features (Wangensteen et al., 2013a, 2013b).

It has been suggested that the tropicalization of NW Mediterranean can lead to a shift in dominance between the temperate common sea urchin *Paracentrotus lividus*, which will suffer from warming temperatures, and the thermophilous black sea urchin *A. lixula* (Gianguzza et al., 2011, Wangensteen et al., 2013a,b, Carreras et al., 2020). Such a shift can have drastic ecological impacts, as both species are conspicuous engineer species shaping benthic communities (Bulleri et al., 1999, Bonaviri et al., 2011). Specific biological and genomic studies are needed to understand the adaptive capabilities of *A. lixula* to ongoing warming, but our results add to the available evidence that colder rather than warmer temperatures may be a limiting factor for *A. lixula*. The absence of clear signs of stress at warm temperatures in adults of *A. lixula*, together with information on larvae development and gonad maturation (Wangensteen et al., 2013a and 2013b), support the hypothesis of the positive

effect of winter warming on the species' reproduction output and larval survival. The ongoing expansion of the species across the littoral coast of the Mediterranean, with the concomitant impacts of its grazing activity on littoral communities, may be exacerbated in the near future by rising winter temperatures in the NW Mediterranean.

ACKNOWLEDGEMENTS

We are indebted to José Carrillo Ortiz, María Casso, Oriol Sacristan, Magdalena Guardiola, and Vanessa Arranz for helping during the thermal stress experiments. This research was funded by the Spanish Government projects ADAPTIVE PGC2018-100735-B-I00 (MCIU/AEI/FEDER, UE) and PopCOmics CTM2017-88080 (MCIU/AEI/FEDER, UE) and *Juan de la Cierva* contracts to RPP and AR.

REFERENCES

Agnello, M., & Roccheri, M. C. (2010). Apoptosis: focus on sea urchin development. *Apoptosis 15*(3), 322-330.

Anestis, A., Lazou, A., Pörtner, H. O., & Michaelidis, B. (2007). Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient temperature. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 293*, R911-R921.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research*, *25*, 3389-3402.

Ardley, H.C. & Robinson, P.A. (2005). E3 ubiquitin ligases. *Essays in Biochemistry*, *41*, 15–30.

Baird, C. H., Niederlechner, S., Beck, R., Kallweit, A. R., & Wischmeyer, P. E. (2013). L-Threonine induces heat shock protein expression and decreases apoptosis in heat-stressed intestinal epithelial cells. *Nutrition*, *29*, 1404-1411.

Basile, A., Biziato, D., Sherbet, G. V., Comi, P., & Cajone, F. (2008). Hyperthermia inhibits cell proliferation and induces apoptosis: relative signaling status of P53, S100A4, and Notch in heat sensitive and resistant cell lines. *Journal of cellular biochemistry*, *103*, 212-220.

Beck, G., O'Brien, R.F., Habicht, G.S., Stillman, D.L., Cooper, E.L., & Raftos, D.A. (1993). Invertebrate cytokines III: Invertebrate interleukin-1-like molecules stimulate phagocytosis by tunicate and echinoderm cells. *Cellular Immunology*, *146*, 284-299.

Bitto, A., Lerner, C.A., Nacarelli, T., Crowe, E., Torres, C., & Sell, C. (2014). P62/SQSTM1 at the interface of aging, autophagy, and disease. *Age*, *36*, 9626.

Bonaviri, C., Fernández, T. V., Fanelli, G., Badalamenti, F., & Gianguzza, P. (2011). Leading role of the sea urchin *Arbacia lixula* in maintaining the barren state in southwestern Mediterranean. *Marine Biology*, *158*, 2505.

Briand, F. (Ed.). (2008). Climate warming and related changes in Mediterranean marine biota. In *CIESM Workshop Monographs* (No. 35). CIESM, Monaco.

Buckley, B. A., Gracey, A. Y., & Somero, G. N. (2006). The cellular response to heat stress in the goby *Gillichthys mirabilis*: a cDNA microarray and protein-level analysis. *Journal of Experimental Biology*, *209*, 2660-2677.

Buckley, L. B., & Huey, R. B. (2016). How extreme temperatures impact organisms and the evolution of their thermal tolerance. *Integrative and comparative biology*, *56*, 98-109.

Calosi, P., Melatunan, S., Turner, L. M., Artioli, Y., Davidson, R. L., Byrne, J. J., ... & Rundle, S. D. (2017). Regional adaptation defines sensitivity to future ocean acidification. *Nature communications*, *8*, 13994.

Carreras, C., García-Cisneros, A., Wangensteen, O. S., Ordóñez, V., Palacín, C., Pascual, M., & Turon, X. (2020). East is East and West is West: Population genomics and hierarchical analyses reveal genetic structure and adaptation footprints in the keystone species *Paracentrotus lividus* (Echinoidea). *Diversity and Distributions*, *26*, 382-398.

Cerrano, C., Bavestrello, G., Bianchi, C. N., Cattaneo-Vietti, R., Bava, S., Morganti, C., … & Siccardi, A. (2000). A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. *Ecology letters*, *3*, 284-293.

Christie, D. L. (2007). Functional insights into the creatine transporter. In *Creatine and creatine kinase in health and disease* (pp. 99-118). Springer, Dordrecht.

Clark, M. S., Suckling, C. C., Cavallo, A., Mackenzie, C. L., Thorne, M. A., Davies, A. J., & Peck, L. S. (2019). Molecular mechanisms underpinning transgenerational plasticity in the green sea urchin *Psammechinus miliaris*. *Scientific reports*, *9*, 952.

Claudet, J., & Fraschetti, S. (2010). Human-driven impacts on marine habitats: a regional meta-analysis in the Mediterranean Sea. *Biological Conservation*, *143*, 2195-2206.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., et al. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS One* 5:e11842

Coma, R., Ribes, M., Serrano, E., Jiménez, E., Salat, J., & Pascual, J. (2009). Global warming-enhanced stratification and mass mortality events in the Mediterranean. *Proceedings of the National Academy of Sciences*, *106*, 6176-6181.

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics. 21*, 3674–3676

765 Cross, T. G., Scheel-Toellner, D., Henriquez, N. V., Deacon, E., Salmon, M., & Lord, J. M. (2000).
766 Serine/threonine protein kinases and apoptosis. *Experimental cell research*, *256*, 34-41.
767
768 De Caralt, S., González, J., Turon, X., & Uriz, J.M. (2018). Reproductive strategies of two common
769 sympatric Mediterranean sponges: *Dysidea avara* (Dictyoceratida) and *Phorbas tenacior*
770 (Poecilosclerida). *PeerJ* 6:e5458
771
772 De Lima Morais, D. A., Fang, H., Rackham, O. J. L., Wilson, D., Pethica, R., Chothia, C., & Gough,
773 J. (2011) SUPERFAMILY 1.75 including a domain centric gene ontology method. *Nucleic Acids*
774 *Research, 39,* D427–D434.
775
776 Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H. O., & Huey, R. B. (2015). Climate change tightens a
777 metabolic constraint on marine habitats. *Science*, *348*, 1132-1135.
778
779 Di Natale, M., Bennici, C., Biondo, G., Masullo, T., Monastero, C., Tagliavia, M., ... & Nicosia, A.
780 (2019). Aberrant gene expression profiles in Mediterranean sea urchin reproductive tissues after metal
781 exposures. *Chemosphere*, *216*, 48-58.
782
783 Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R.,
784 Munday PL (2019). Understanding interactions between plasticity, adaptation and range shifts in
785 response to marine environmental change. *Philosophical Transactions of the Royal Society B*, 374,
786 20180186.
787
788 Dong, Y. W., Yu, S. S., Wang, Q. L., & Dong, S. L. (2011). Physiological responses in a variable
789 environment: relationships between metabolism, hsp and thermotolerance in an intertidal-subtidal
790 species. *PLoS One*, *6*, e26446.
791
792 Elmasry, E., Razek, F. A. A., El-Sayed, A. F. M., Omar, H., & El Sayed, A. E. (2015). Abundance,
793 size composition and benthic assemblages of two Mediterranean echinoids off the Egyptian coasts:
794 *Paracentrotus lividus* and *Arbacia lixula*. *The Egyptian Journal of Aquatic Research*, *41*, 367-374.
795
796 Evans, T. G., Pespeni, M. H., Hofmann, G. E., Palumbi, S. R., & Sanford, E. (2017). Transcriptomic
797 responses to seawater acidification among sea urchin populations inhabiting a natural pH mosaic.
798 *Molecular ecology*, *26*, 2257-2275.
799
800 Feder, M. E., & Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress
801 response: evolutionary and ecological physiology. *Annual review of physiology*, *61*, 243-282.
802
803 Feder, M. E., & Walser, J. C. (2005). The biological limitations of transcriptomics in elucidating stress
804 and stress responses. *Journal of evolutionary biology*, *18*, 901-910.
805
806 Francour, P., Boudouresque, C. F., Harmelin, J. G., Harmelin-Vivien, M. L., & Quignard, J. P. (1994).
807 Are the Mediterranean waters becoming warmer? Information from biological indicators. *Marine*
808 *Pollution Bulletin*, *28*, 523-526.
809
810 Gaitán-Espitia, J. D., Sánchez, R., Bruning, P., & Cárdenas, L. (2016). Functional insights into the
811 testis transcriptome of the edible sea urchin *Loxechinus albus*. *Scientific reports*, *6*, 36516.
812
813 Garcia-Cisneros, A., Palacín, C., Ventura, C. R. R., Feital, B., Paiva, P. C., & Pérez-Portela, R. (2018).
814 Intraspecific genetic structure, divergence and high rates of clonality in an amphi-Atlantic starfish.
815 *Molecular ecology*, *27*, 752-772.
816

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., ... & Ledoux, J. B. (2009). Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global change biology*, *15*, 1090-1103.

Gianguzza, P., Agnetta, D., Bonaviri, C., Di Trapani, F., Visconti, G., Gianguzza, F., & Riggio, S. (2011) The rise of thermophilic sea urchins and the expansion of barren grounds in the Mediterranean Sea. *Chemistry and Ecology*, *27*, 129-134.

Gianguzza, P., Visconti, G., Gianguzza, F., Vizzini, S., Sarà, G., & Dupont, S. (2014). Temperature modulates the response of the thermophilous sea urchin *Arbacia lixula* early life stages to CO2-driven acidification. *Marine environmental research*, *93*, 70-77.

Gierz, S. L., Forêt, S., & Leggat, W. (2017). Transcriptomic analysis of thermally stressed Symbiodinium reveals differential expression of stress and metabolism genes. *Frontiers in plant science*, *8*, 271.

Gillard, G. B., Garama, D. J., & Brown, C. M. (2014). The transcriptome of the NZ endemic sea urchin Kina (*Evechinus chloroticus*). *BMC genomics*, *15*, 45.

González, K., Gaitán-Espitia, J., Font, A., Cárdenas, C. A., & González-Aravena, M. (2016). Expression pattern of heat shock proteins during acute thermal stress in the Antarctic sea urchin, *Sterechinus neumayeri*. *Revista chilena de historia natural*, *89*, 2.

Grabherr M.G., Haas B.J., Yassour M., Levin J.Z., Thompson D.A., Amit I., … Regev, A. (2011). Trinity: reconstructing a full-length transcriptome without a genome from RNA-Seq data. *Nature Biotechnology*. *29*, 644–652

Harmelin, J.G., Hereu, B., De Maisonnave, L.M., Teixidor, N.,Domínguez, L., & Zabala, M. (1995). Indicateurs de biodiversité enmilieu marin: les échinodermes. Fluctuations temporelles des peuplements d'échinodermes à Port-Cros. Comparaison entre les années 1982-84 et 1993-95.Internal Report. Port Cros National Park

Hereu, B., Linares, C., Sala, E., Garrabou, J., Garcia-Rubies, A., Diaz, D., & Zabala, M. (2012). Multiple processes regulate long-term population dynamics of sea urchins on Mediterranean rocky reefs. *PLoS One*, *7*, e36901.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The impact of climate change on the world's marine ecosystems. *Science*, *328*, 1523-1528.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., ... & Bridge, T. C. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, *543*, 373.

Jia, Z., Wang, Q., Wu, K., Wei, Z., Zhou, Z., & Liu, X. (2017). De novo transcriptome sequencing and comparative analysis to discover genes involved in ovarian maturity in *Strongylocentrotus nudus*. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *23*, 27-38.

Jonas, K., Liu, J., Chien, P., & Laub, M.T. (2013). Proteotoxic stress induces a cell cycle arrest by stimulating Lon to degrade the replication initiator DnaA. *Cell*, *154*, 623–636.

Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., ... & Hunter, S. (2014). InterProScan 5: genome-scale protein function classification. *Bioinformatics*, *30*, 1236-1240.

Jordà, G., Marbà, N., & Duarte, C. M. (2012). Mediterranean seagrass vulnerable to regional climate warming. *Nature Climate Change*, *2*, 821.

Kim, B. M., Kim, K., Choi, I. Y., & Rhee, J. S. (2017). Transcriptome response of the Pacific oyster, *Crassostrea gigas* susceptible to thermal stress: A comparison with the response of tolerant oyster. *Molecular & Cellular Toxicology*, *13*(1), 105-113.

King, N. G., McKeown, N. J., Smale, D. A., & Moore, P. J. (2018). The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography*, *41*, 1469-1484.

Kostova, Z., & Wolf, D. H. (2003). For whom the bell tolls: protein quality control of the endoplasmic reticulum and the ubiquitin–proteasome connection. *The EMBO journal*, *22*, 2309-2317.

Langmead, B. & Salzberg, S.L. (2912). Fast gapped-read alignment with Bowtie 2. *Nature Methods* 9, 357–359

Lee, E.W., Lee, M.S., Camus, S. *et al.* (2009). Differential regulation of p53 and p21 by MKRN1 E3 ligase controls cell cycle arrest and apoptosis. *The EMBO Journal*, *28*, 2100–2113.

Lejeusne C, Chevaldonné P, Pergent-Martini C, Boudouresque CF, Pérez T (2010) Climate change effects on a miniature ocean: the highly diverse, highly impacted Mediterranean Sea. *Trends in Ecology and Evolution 25*, 250–260

Lesser, M. P. (2012). Oxidative stress in tropical marine ecosystems. *Oxidative Stress in Aquatic Ecosystems*, *1*, 9-19.

Li, B. & Dewey, C.N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*, *12*, 323

Li, H., & Stillman, B. (2012). The origin recognition complex: a biochemical and structural view. In *The Eukaryotic Replisome: A Guide to Protein Structure and Function* (pp. 37-58). Springer, Dordrecht.

Lodder, A. L., Lee, T. K., & Ballester, R. (1999). Characterization of the Wsc1 protein, a putative receptor in the stress response of *Saccharomyces cerevisiae*. *Genetics*, *152*, 1487-1499

Logan, C. A., & Somero, G. N. (2011). Effects of thermal acclimation on transcriptional responses to acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *300*, R1373-R1383.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550.

Maier, T., Güell, M., & Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. *FEBS letters*, *583*, 3966-3973.

Malhotra, J. D., & Kaufman, R. J. (2007). Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword?. *Antioxidants & redox signaling*, *9*, 2277-2294.

Marbà, N., Jordà, G., Agustí, S., Girard, C., & Duarte, C. M. (2015). Footprints of climate change on Mediterranean Sea biota. *Frontiers in Marine Science*, *2*, 56.

Martins, C. S., Hamann, M., & Fiúza, A. F. (2002). Surface circulation in the eastern North Atlantic, from drifters and altimetry. *Journal of Geophysical Research: Oceans*, *107*, 10-1.

Matranga, V., Toia, G., Bonaventura, R., & Müller, W. E. (2000). Cellular and biochemical responses to environmental and experimentally induced stress in sea urchin coelomocytes. *Cell stress & chaperones*, *5*, 113.

Matranga, V., Bonaventura, R., & Di, G. B. (2002). Hsp70 as a stress marker of sea urchin coelomocytes in short term cultures. *Cellular and molecular biology (Noisy-le-Grand, France)*, *48*, 345-349.

Matranga, V., Pinsino, A., Celi, M., Natoli, A., Bonaventura, R., Schröder, H. C., & Müller, W. E. G. (2005). Monitoring chemical and physical stress using sea urchin immune cells. In *Echinodermata* (pp. 85-110). Springer, Berlin, Heidelberg.

Mosser, D. D., Caron, A. W., Bourget, L., Meriin, A. B., Sherman, M. Y., Morimoto, R. I., & Massie, B. (2000). The chaperone function of hsp70 is required for protection against stress-induced apoptosis. *Molecular and cellular biology*, *20*, 7146-7159.

Ngosuwan, J., Wang, N. M., Fung, K. L., & Chirico, W. J. (2003). Roles of cytosolic *Hsp70* and *Hsp40* molecular chaperones in post-translational translocation of presecretory proteins into the endoplasmic reticulum. *Journal of Biological Chemistry*, *278*, 7034-7042.

Oleksiak, M. F., Roach, J. L., & Crawford, D. L. (2005). Natural variation in cardiac metabolism and gene expression in *Fundulus heteroclitus*. *Nature genetics*, *37*, 67.

Oliver, E. C., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., ... & Holbrook, N. J. (2018). Longer and more frequent marine heatwaves over the past century. *Nature communications*, *9*, 1324.

Ordóñez, V., Pascual, M., Fernández-Tejedor, M. Pineda, M.C., Tagliapietra, D., Turon, X. (2015). Ongoing expansion of the worldwide invader *Didemnum vexillum* (Ascidiacea) in the Mediterranean Sea: high plasticity of its biological cycle promotes establishment in warm waters**.** *Biological Invasions 17*, 2075-2085.

Osovitz CJ, Hofmann GE (2005). Thermal history-dependent expression of the hsp70 gene in purple sea urchin: Biogeographic patterns and the effect of temperature acclimation. *Journal of Experimental Marine Biology and Ecology*, *327*, 134-143.

Ozato, K., Shin, D. M., Chang, T. H., & Morse III, H. C. (2008). TRIM family proteins and their emerging roles in innate immunity. *Nature reviews immunology*, *8*, 849.

Palacín, C., Turon, X., Ballesteros, M., Giribet, G., & López, S. (1998). Stock Evaluation of three littoral echinoid species on the Catalan Coast North-Western Mediterranean. *Marine Ecology*, *19*, 163-177.

Parry, M. L., Canziani, O. F., Palutikof, J. P., Van Der Linden, P. J., & Hanson, C. E. (2007). IPCC, 2007: climate change 2007: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change. *Cambridge Uni-versity Press, Cambridge, UK*.

Pastor, F. J. (2012). Ciclogénesis intensas en la cuenca occidental del Mediterráneo y temperatura superficial del mar: Modelización y evaluación de las áreas de recarga. *PhD dissertation.* https://www.tdx.cat/handle/10803/83620

Pérez-Portela, R., & Riesgo, A. (2013). Optimizing preservation protocols to extract high-quality RNA from different tissues of echinoderms for next generation sequencing. *Molecular ecology resources*, *13*, 884-889.

Pérez-Portela, R., Turon, X., & Riesgo, A. (2016). Characterization of the transcriptome and gene expression of four different tissues in the ecologically relevant sea urchin *Arbacia lixula* using RNA-seq. *Molecular ecology resources*, *16*, 794-808.

Pérez-Portela, R., Wangensteen, O. S., Garcia-Cisneros, A., Valero-Jiménez, C., Palacín, C., & Turon, X. (2019). Spatio-temporal patterns of genetic variation in *Arbacia lixula*, a thermophilous sea urchin in expansion in the Mediterranean. *Heredity*, *122*, 244.

Pinsino, A., Della Torre, C., Sammarini, V., Bonaventura, R., Amato, E., & Matranga, V. (2008). Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: a field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy. *Cell biology and toxicology*, *24*, 541-552.

Pörtner, H. O. (2002). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *132*, 739-761.

Privitera, D., Noli,M., Falugi, C., Chiantore,M. (2011). Benthic assemblages and temperatura effects on *Paracentrotus lividus* and *Arbacia lixula* larvae and settlement. *Journal of Experimental Marine Biology and Ecology*, *407*(1), 6-11.

Prusina, I., Sarà, G., De Pirro, M., Dong, Y. W., Han, G. D., Glamuzina, B., Williams, G. A. (2014). Variations in physiological responses to thermal stress in congeneric limpets in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology*, *456*, 34-40.

Rachel, A., Tyson, J. R., & Stirling, C. J. (1997). A novel subfamily of Hsp70s in the endoplasmic reticulum. *Trends in cell biology*, *7*, 277-282.

Raitsos, D. E., Beaugrand, G., Georgopoulos, D., Zenetos, A., Pancucci-Papadopoulou, A. M., Theocharis, A., & Papathanassiou, E. (2010). Global climate change amplifies the entry of tropical species into the Eastern Mediterranean Sea. *Limnology and Oceanography*, *55*, 1478-1484.

Rao, R. V., Peel, A., Logvinova, A., del Rio, G., Hermel, E., Yokota, T., ... & Bredesen, D. E. (2002). Coupling endoplasmic reticulum stress to the cell death program: role of the ER chaperone GRP78. *FEBS letters*, *514*, 122-128.

Rilov G (2016) Multi-species collapses at the warm edge of the warming sea. *Scientific reports*, *6*, 36897.

Runcie, D. E., Garfield, D. A., Babbitt, C. C., Wygoda, J. A., Mukherjee, S., & Wray, G. A. (2012). Genetics of gene expression responses to temperature stress in a sea urchin gene network. *Molecular ecology*, *21*, 4547-4562.

Schoville, S. D., Barreto, F. S., Moy, G. W., Wolff, A., & Burton, R. S. (2012). Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod *Tigriopus californicus*. *BMC evolutionary biology*, *12* 170.

Shaltout, M., & Omstedt, A. (2014). Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. *Oceanologia*, *56*, 411-443.

Shental-Bechor, D., & Levy, Y. (2008). Effect of glycosylation on protein folding: a close look at thermodynamic stabilization. *Proceedings of the National Academy of Sciences*, *105*, 8256-8261.

Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V. & Zdobnov, E.M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210-3212.

Smale, D. A., Wernberg, T., Oliver, E. C., Thomsen, M., Harvey, B. P., Straub, S. C., ... & Feng, M. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, *9*, 306.

Smith, L. C., Rast, J. P., Brockton, V., Terwilliger, D. P., Nair, S. V., Buckley, K. M., & Majeske, A. J. (2006). The sea urchin immune system. *Invertebrate Survival Journal*, *3*, 25-39.

Smith, L. C., Arriza, V., Hudgell, M. A. B., Barone, G., Bodnar, A. G., Buckley, K. M., ... & Furukawa, R. (2018). Echinodermata: The Complex Immune System in Echinoderms. In *Advances in Comparative Immunology* (pp. 409-501). Springer, Cham.

Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology*, *213*, 912-920.

Somot, S., Sevault, F., & Déqué, M. (2006). Transient climate change scenario simulation of the Mediterranean Sea for the twenty-first century using a high-resolution ocean circulation model. *Climate Dynamics*, *27*, 851-879.

Somot, S., Sevault, F., Déqué, M., & Crépon, M. (2008) 21st century climate change scenario for the Mediterranean using a coupled atmosphere– ocean regional climate model. *Global Planet Change*, *63*, 112–126

Stapley, J., Reger, J., Feulner, P. G., Smadja, C., Galindo, J., Ekblom, R., ... & Slate, J. (2010). Adaptation genomics: the next generation. *Trends in ecology & evolution*, *25*, 705-712.

Stillman, J. H. (2019). Heat waves, the new normal: summertime temperature extremes will impact animals, ecosystems, and human communities. *Physiology*, *34*, 86-100.

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, *301*, 65-65.

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One*, *6*, e21800.

Templado, J. (2014). Future trends of Mediterranean biodiversity. In *The Mediterranean Sea* (pp. 479-498). Springer, Dordrecht.

Tomanek, L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *Journal of Experimental Biology*, *213*, 971-979.

Tortonese, E. (Ed.). (1965). *Fauna d'Italia: Echinodermata*. Ed. Calderini.

Visconti, G., Gianguzza, F., Butera, E., Costa, V., Vizzini, S., Byrne, M., & Gianguzza, P. (2017). Morphological response of the larvae of Arbacia lixula to near-future ocean warming and acidification. *ICES Journal of Marine Science*, *74*, 1180-1190.

Wang, F., Yang, H., Gao, F., & Liu, G. (2008). Effects of acute temperature or salinity stress on the immune response in sea cucumber, *Apostichopus japonicus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *151*, 491-498.

Wangensteen, O.S., Turon, X., García-Cisneros, A., Recasens, M., Romero, J., & Palacín, C. (2011). A wolf in sheep's clothing: carnivory in dominant sea urchins in the Mediterranean. *Marine Ecology Progress Series*, *441*, 117–128.

Wangensteen, O. S., Turon, X., Pérez-Portela, R., & Palacín, C. (2012). Natural or naturalized? Phylogeography suggests that the abundant sea urchin *Arbacia lixula* is a recent colonizer of the Mediterranean. *PLoS One*, *7*, e45067.

Wangensteen, O. S., Dupont, S., Casties, I., Turon, X., & Palacín, C. (2013a). Some like it hot: temperature and pH modulate larval development and settlement of the sea urchin *Arbacia lixula*. *Journal of experimental marine biology and ecology*, *449*, 304-311.

Wangensteen, O. S., Turon, X., Casso, M., & Palacín, C. (2013b). The reproductive cycle of the sea urchin *Arbacia lixula* in northwest Mediterranean: potential influence of temperature and photoperiod. *Marine biology*, *160*, 3157-3168.

Warnes, M. G. R., Bolker, B., Bonebakker, L., & Gentleman, R. (2016). Package 'gplots'. *Various R Programming Tools for Plotting Data*.

Xu, D., Zhou, S., & Sun, L. (2018). RNA-seq based transcriptional analysis reveals dynamic genes expression profiles and immune-associated regulation under heat stress in *Apostichopus japonicus*. *Fish & shellfish immunology*, *78*, 169-176.

Yao, C. L., & Somero, G. N. (2012). The impact of acute temperature stress on hemocytes of invasive and native mussels (*Mytilus galloprovincialis* and *Mytilus californianus*): DNA damage, membrane integrity, apoptosis and signaling pathways. *Journal of Experimental Biology*, *215*, 4267-4277.

Young, C. M., Tyler, P. A., & Fenaux, L. (1997). Potential for deep sea invasion by Mediterranean shallow water echinoids: pressure and temperature as stage-specific dispersal barriers. *Marine Ecology Progress Series*, *154*, 197-209.

Zhao, C., Zhang, L., Shi, D., Ding, J., Yin, D., Sun, J., ... & Chang, Y. (2018). Transgenerational effects of ocean warming on the sea urchin *Strongylocentrotus intermedius*. *Ecotoxicology and environmental safety*, *151*, 212-219.

Zheng, J., Cao, J., Mao, Y., Su, Y., & Wang, J. (2019). Comparative transcriptome analysis provides comprehensive insights into the heat stress response of *Marsupenaeus japonicus*. *Aquaculture*, *502*, 338-346.

Zhu, Q., Zhang, L., Li, L., Que, H., & Zhang, G. (2016). Expression characterization of stress genes under high and low temperature stresses in the Pacific oyster, *Crassostrea gigas*. *Marine biotechnology*, *18*, 176-188.

DATA ACCESSIBILITY

The *De novo* assemblies, RSEM, annotation and DEseq files are available at Mendeley Data doi.org/10.17632/5673n552yj.1.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of this study and were involved in the aquarium experiments. RPP, AR, XT and OSW analysed the data. RPP wrote the first draft of the manuscript and created figures and tables. XT, AR and CP contributed to improve the first draft, and all authors revised the final version of the manuscript.

FIGURE LEGENDS

Figure 1. Sampling and experiments in *Arbacia lixula*. A- Samples analysed for gene expression: Comparison between Control condition (CT at 13°C) and temperature at 7°C (CONTROL *vs* T7), and Control and 22°C (CONTROL *vs* T22). Red crosses indicated replicates lost during the development of the experiments, and B- Map of the sampling area of *A. lixu*la.

Figure 2. Pipeline followed in this study. The most important experimental steps and analyses are represented.

Figure 3. Number of differentially expressed (DE) transcripts between treatments and experiments. A- Comparison of number and percentage of up- and down- regulated transcripts between treatments at each experiment, and B- Venn diagram representing the number of DE transcripts per experiment and those (14) in common between experiments.

Figure 4. Heatmaps based on differentially expressed transcripts (DE) from pairwise comparisons of treatments within experiments. A- Control condition versus Temperature 7°C, and B- Control condition versus Temperature 22°C. Different colours indicate relative expression levels. Similarity in gene expression patterns among replicates (individuals) is represented by clustering on the top of the heatmaps.

Figure 5. Principal Component Analyses (PCAs) plots for the two different experiments including all replicates per treatment. A- "Control versus Temperature 7°C", and B- "Control versus Temperature 22°C"

Figure 6. Gene Ontology treemaps for annotated differentially expressed genes in Control versus Temperature 7°C. DOWN- and UP- regulated categories at 7°C are presented as separated figures for Biological Processes, Cellular components, and Molecular functions. The size of the rectangles reflects the log2foldchage associated to the differentially regulated categories.
[**1**= multicellular organism development; **2**= positive regulation of GTPase activity; **3**= regulation of ARF protein signal transduction; **4** single−organism cellular process**; 5**= DNA replication; **6**= polyamine biosynthetic process; **7**= histone H3 deacetylation; **8**= DNA−templated transcription initiation; **9**= microtubule−based movement; **10**= mitochondrion organization; **11**= microtubule motor activity; **12**= calcium ion binding; **13**= pantothenate biosynthetic process; **14**= microtubule−based

process; **15**= RNA secondary structure unwinding; **16**= oxidation-reduction process; **17**= heterocyclic compound binding; **18**= organic cyclic compound binding; **19**= sequence−specific DNA Binding; **20**= poly(A) RNA Binding; **21**= heme binding; **22**= methyltransferase Activity; **23**: protein kinase activity; **24**= monooxygenase activity; **25**= oxidoreductase activity; **26**= peptidyl−prolyl cis−transisomerase activity; **27**= G−protein coupled receptor activity; **28**= oxidoreductase activity; **29**= structural constituent of cytoskeleton; **30**= protein heterodimerization activity; **31**= transcription factor activity, sequence−specific DNA binding; **32**= phosphatidylserine decarboxylase activity; **33**= protein phosphatase regulator activity]

Figure 7. Gene Ontology treemaps for annotated differentially expressed genes in Control versus Temperature 22°C. Only the function of DOWN- regulated genes at 22°C was obtained for Biological Processes, Cellular components, and Molecular functions. The size of the rectangles reflects the log2foldchage associated to the differentially regulated categories.

Reference	Reads	Genes	Transc.	N50	N20	Blast hit	GC%	M_L	Avg_L	Mb
CT+T7	341,735,712	151,418	211,456	1,102	2,960	41,429	41.68	345	668.6	141.5
CT+T22	351,275,576	151,278	215,362	1,114	2,976	38,691	41.41	345	671.1	147.4

Table 1. General statistics of the reference assemblies CT_T7 and CT_T22 of *A. lixula*. Total number of trimmed reads assembled (Reads), number of genes, number of transcripts (Transc.: including genes and isoforms collapsed into genes), parameters N50 and N20, number of transcripts with Blast hit against proteins of metazoans, percentage of GC (GC%), median transcript length (M_L), average transcript length (Avg_L), and number of assembled bases expressed as Mb.

Treatment	Replicate	Gender	Nº Reads
7ºC	1	F	74,582,590
	2	M	40,645,290
	3	F	83,661,578
	4	M	60,222,882
	5	M	140,478,920
Control	1	F	41,330,158
	2	M	84,053,170
	3	M	23,074,766
	4	F	59,854,444
	5	F	26,843,788
	6	M	48,723,838
22ºC	1	F	50,432,902
	2	M	36,718,372
	3	F	139,884,462
	4	M	103,939,816
	6	M	87,695,436

Table 2. Treatment and replicate, gender and number of trimmed reads used for differential expression analyses. Note that replicates 6 and 5 from 7ºC and 22ºC, respectively, are missing due to low quality of the libraries.

Eukarya						
Reference	**C**	**S**	**D**	**F**	**M**	**Groups searched**
CT+T7	290 (95.7%)	203 (67%)	87 (28.7%)	12 (4%)	1 (0.3%)	
CT+T22	286 (94.4%)	206 (68%)	80 (26.4%)	16 (5.3%)	1 (0.3%)	303
Metazoa						
Reference	**C**	**S**	**D**	**F**	**M**	**Groups searched**
CT+T7	924 (94.5%)	618 (63.2%)	306 (31.3%)	45 (4.6%)	9 (0.9%)	
CT+T22	925 (94.6%)	603 (61.7%)	322 (32.9%)	42 (4.3%)	11 (1.1%)	978

Table 3. Number and percentage of BUSCO groups recovered in the searches against the eukaryotic and metazoan databases. Complete BUSCOs (C), complete and single-copy BUSCOs (S), complete and duplicated BUSCOs (D), fragmented BUSCOs (F), and missing BUSCOs (M).

Experiment	**DE transcripts**	**Blast hit**	**GO term**
Control vs T7	1181	445	84
Control vs T22	179	35	3

Table 4. Differentially expressed (DE) transcripts between treatments. Number of DE transcripts between treatments for each experiment, number of DE transcripts with blast hit against metazoan proteins per experiment, and number of DE transcripts with an associated GO term per treatment.

Figure 1. Sampling and experiments in *Arbacia lixula*. A- Samples analysed for gene expression: Comparison between Control condition (CT at 13°C) and temperature at 7°C (CONTROL *vs* T7), and Control and 22°C (CONTROL *vs* T22). Red crosses indicated replicates lost during the development of the experiments, and B- Map of the sampling area of *A. lixu*la.

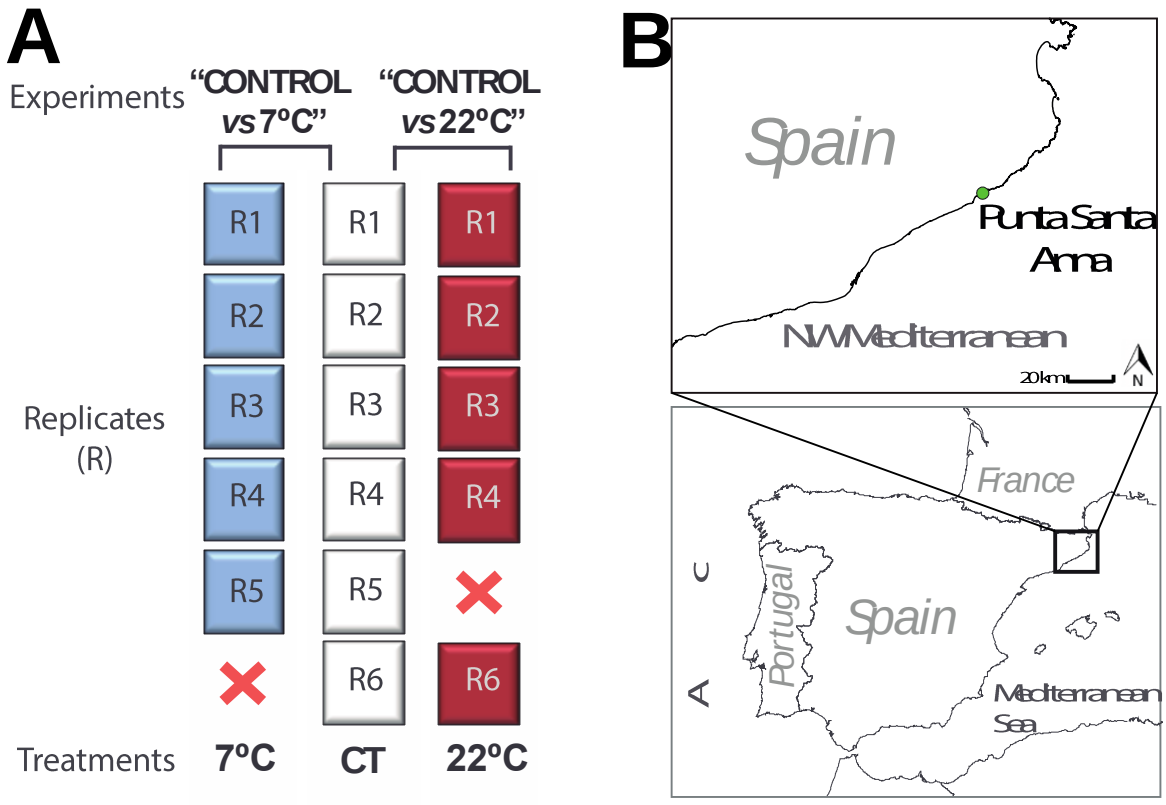


Figure 2. Pipeline followed in this study. The most important experimental steps and analyses are represented.

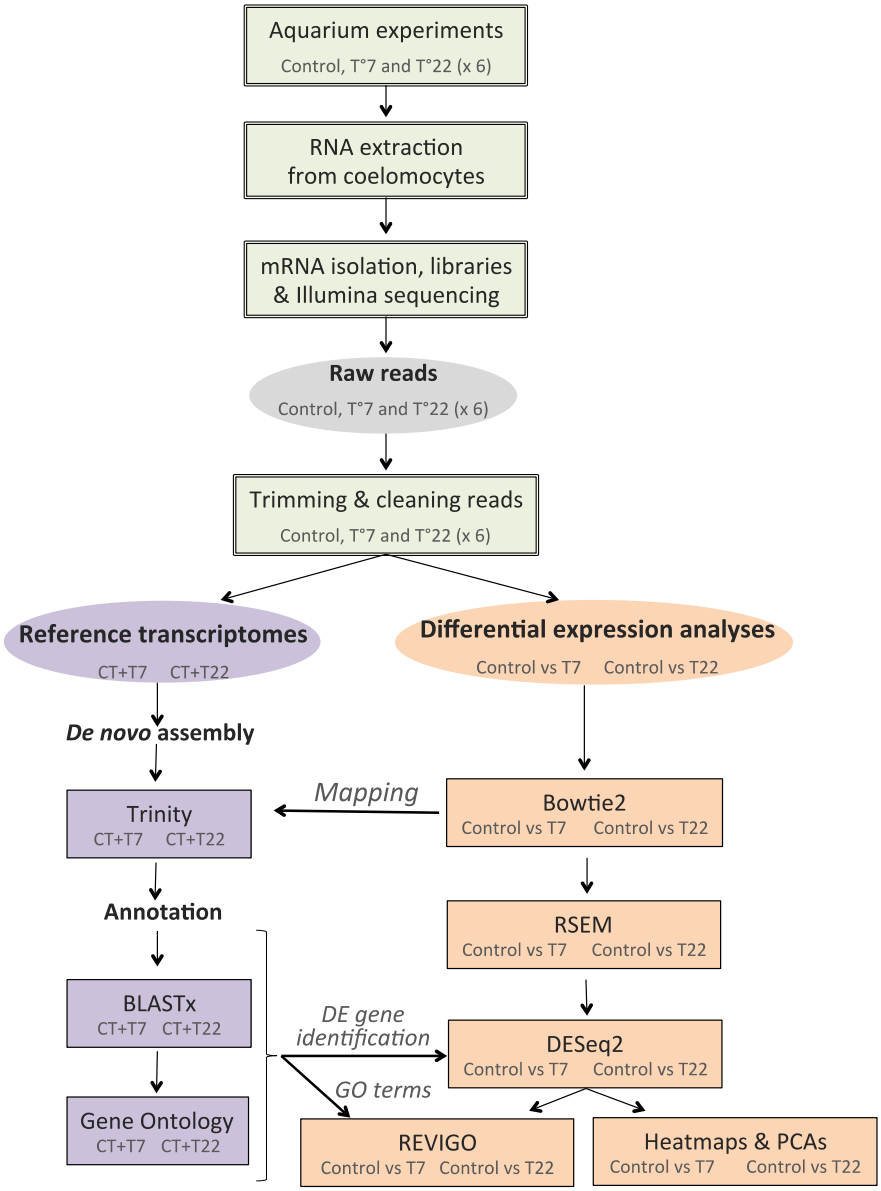


Figure 3. Number of differentially expressed (DE) transcripts between treatments and experiments. A- Comparison of number and percentage of up- and down- regulated transcripts between treatments at each experiment, and B- Venn diagram representing the number of DE transcript per experiment and those (14) in common between experiments

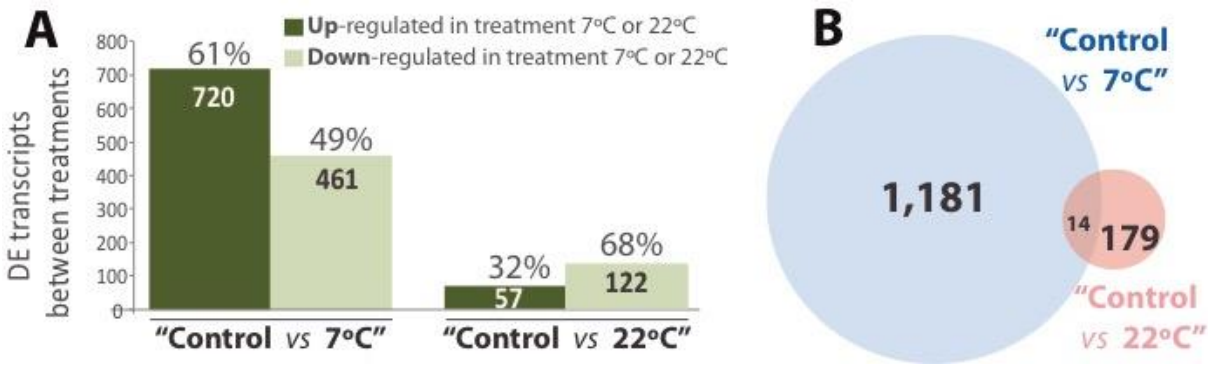


Figure 4. Heatmaps based on differentially expressed transcripts (DE) from pairwise comparisons of treatments within experiments. A- Control condition versus Temperature 7°C, and B- Control condition versus Temperature 22°C. Different colours indicate relative expression levels. Similarity in gene expression patterns among replicates (individuals) is represented by clustering on the top of the heatmaps.

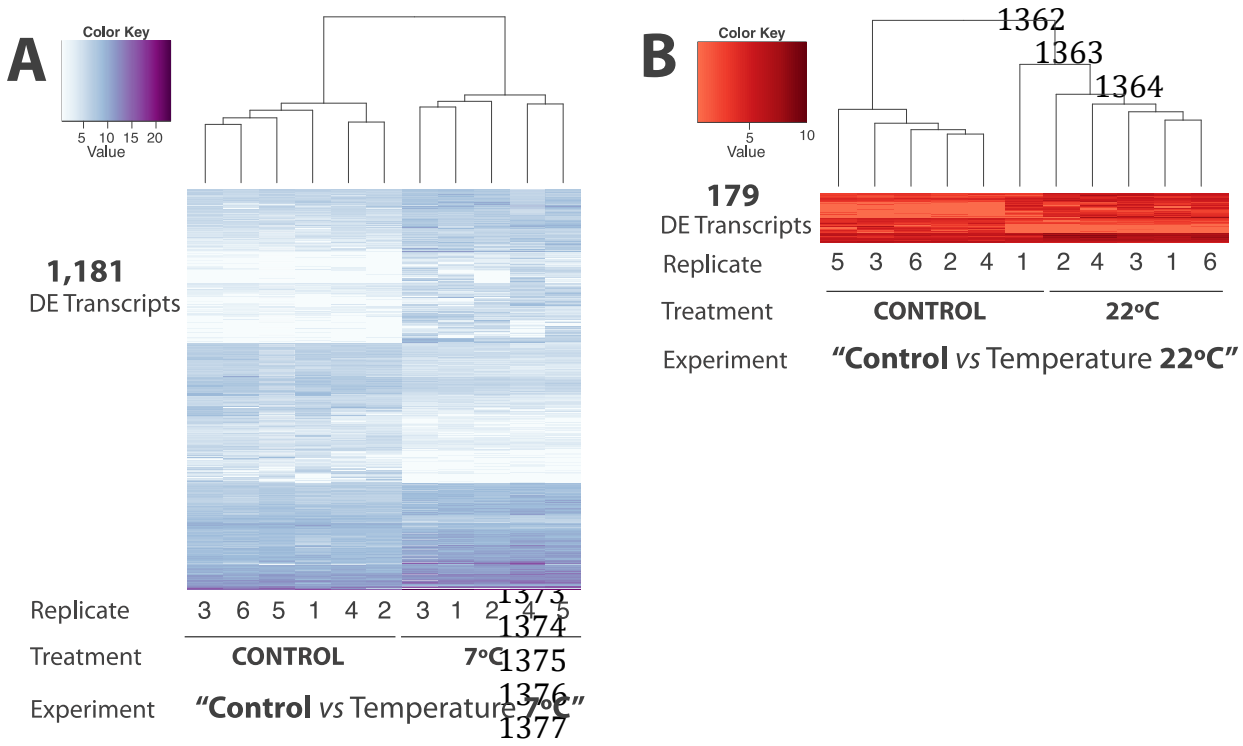


Figure 5. Principal Component Analyses (PCAs) plots for the two different experiments including all replicates per treatment. A- “Control versus Temperature 7°C”, and B- “Control versus Temperature 22°C”

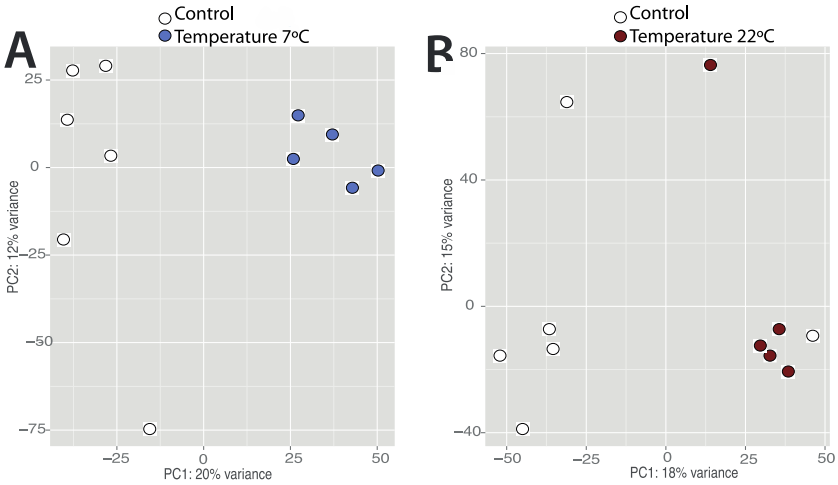
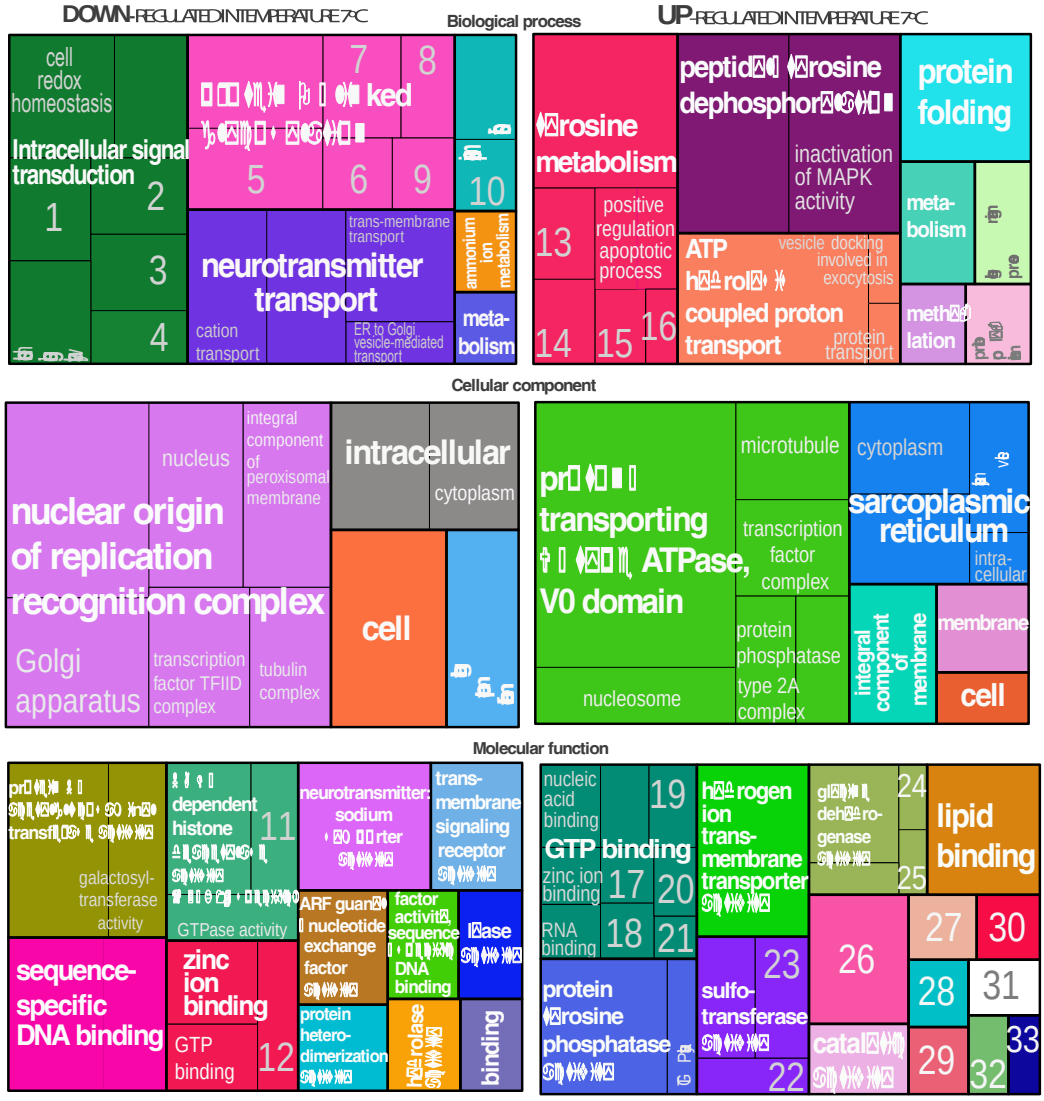


Figure 6. Gene Ontology treemaps for annotated differentially expressed genes in Control versus Temperature 7°C. DOWN- and UP- regulated categories at 7°C are presented as separated figures for Biological Processes, Cellular components, and Molecular functions. The size of the rectangles reflects the log2foldchage associated to the differentially regulated categories.



[**1**= multicellular organism development; **2**= positive regulation of GTPase activity; **3**= regulation of ARF protein signal transduction; **4** single−organism cellular process**;** **5**= DNA replication; **6**= polyamine biosynthetic process; **7**= histone H3 deacetylation; **8**= DNA−templated transcription initiation; **9**= microtubule−based movement; **10**= mitochondrion organization; **11**= microtubule motor activity; **12**= calcium ion binding; **13**= pantothenate biosynthetic process; **14**= microtubule−based process; **15**= RNA secondary structure unwinding; **16**= oxidation-reduction process; **17**= heterocyclic compound binding; **18**= organic cyclic compound binding; **19**= sequence−specific DNA Binding; **20**= poly(A) RNA Binding; **21**= heme binding; **22**= methyltransferase Activity; **23**: protein kinase activity; **24**= monooxygenase activity; **25**= oxidoreductase activity; **26**= peptidyl−prolyl cis−transisomerase activity; **27**= G−protein coupled receptor activity; **28**= oxidoreductase activity; **29**= structural constituent of cytoskeleton; **30**= protein heterodimerization activity; **31**= transcription factor activity, sequence−specific DNA binding; **32**= phosphatidylserine decarboxylase activity; **33**= protein phosphatase regulator activity]

Figure 7. Gene Ontology treemaps for annotated differentially expressed genes in Control versus Temperature 22°C. Only the function of DOWN- regulated genes at 22°C was obtained for Biological Processes, Cellular components, and Molecular functions. The size of the rectangles reflects the log2foldchage associated to the differentially regulated categories between 22°C and the control condition.